ACE İnhibitörlerinin ve Aldosteron Reseptör Blokerlerinin Fibrinolitik Sistem Üzerindeki Etkileriyle İlgili Dinamik Karşılaştırmalı Bir Çalışma

A Dynamic Comparative Study Concerning the Effects of ACE Inhibitors and Aldosterone Receptor Blockers on Fibrinolytic System

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ÖZET

Giriş: Renin-anjiyotensin-aldosteron sistemi (RAAS) fibrinolitik sistemde merkezi rol oynar. RAAS aktivasyonu tromboembolik olayların patofizyolojisinde direkt olarak yer alan plazminojen aktivatör inhibitörünün (PAI-1) ekspresyonunu stimüle eder. Bu çalışmadaki primer amaçlarımız, 1) akut RAAS aktivasyonunun plazma PAI-1 düzeylerine olan etkisini ölçmek ve 2) yalnız başına ACE inhibitörü ve ACE inhibitörü ile kombine spironolaktonun PAI-1 düzeyleri üzerine etkilerini karşılaştırmaktı.

Yöntem: Bu prospektif ve *in vivo* çalışmada RAAS efektif ve fizyolojik bir yol olan flebotomi ile aktive edildi. On yedi prehipertansif durum haricinde başka hastalığı bulunmayan gönüllü kan donörleri çalışmaya dahil edildi. Renin ve PAI-1 seviyeleri flebotomi öncesi ve sonrasında ölçüldü. İkinci flebotomi esnasında 17 donörden 12'si, 6'sı enalapril (5 mg) ve diğer altısı enalapril+spironolakton (25 mg) almak üzere iki gruba ayrıldı; 5 donör ise kontrol grubu olarak seçildi.

Bulgular: Plasma renin ve PAI-1 seviyeleri başlangıç flebotomisini takiben anlamlı olarak arttı. İkinci flebotomi esnasında, birincisine kıyasla plasma PAI-1 aktivitesi anlamlı olarak düştü; ancak bu düşüşün bazal seviyeye ulaşmadığı görüldü. PAI-1 seviyelerindeki ortalama düşüşün kombine medikasyon alan grupta daha fazla olduğu gözlendi.

Sonuç: Enalapril ve enalapril ile kombine spironolakton kullanımı PAI-1 seviyelerinde anlamlı düşüşe yol açmaktadır. Ancak bu düşüş kombine rejimle daha belirgin ortaya çıkmaktadır. Ne var ki, iki rejimde de PAI-1 aktivitesi bazal seviyelere düşmemektedir.

Anahtar sözcükler: flebotomi, renin-anjiyotensin-aldosteron sistem aktivasyonu, PAI-1, enalapril, spironolakton

ABSTRACT

Backround: The renin-angiotensin-aldosterone system plays a central role in fibrinolysis. Activation of the RAAS stimulates the expression of plasminogen activator inhibitor (PAI-1), which can be directly implicated in the pathophysiology of thromboembolic events. Our primary aims were, 1) to measure the effect of acute RAAS activation on plasma levels of PAI-1, and 2) to measure the inhibitory effect of an ACE inhibitor alone, versus a combination of an ACE inhibitor and aldosterone blocker on the usual increase in PAI-1 usually observed.

Methods: In the current prospective *in vivo* study, RAAS was activated by means of phlebotomy, an effective, physiologic means of RAAS activation. Seventeen voluntary pre-hypertensive but otherwise healthy blood donors were included in this study. Renin and PAI-1 levels were measured before and after initial phlebotomy. At the time of the second phlebotomy, 12/17 donors were randomly assigned to receive enalapril (5 mg) or the combination of enalapril (5 mg) plus spironolactone (25 mg), beginning three days prior to phlebotomy and 5 were assigned as controls who received no medications.

Results: Plasma renin and PAI levels were significantly increased following initial phlebotomy. At the time of the second phlebotomy, plasma PAI-1 activity was reduced significantly as compared to the initial phlebotomy, but it did not return to baseline levels. The observed mean reduction in PAI-1 level was greater for the subjects receiving both ACE and aldosterone inhibition.

Conclusion: Enalapril and enalapril plus spironolactone efficiently reduce PAI-1 levels, but the reductions are more pronounced with the combined regimen. However, neither treatment appears sufficient to return PAI-1 activity to baseline levels.

Keywords: phlebotomy, RAAS activation, PAI-1, enalapril, spironolactone

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Introduction

Independent of blood pressure, activation of the renin-angiotensin-aldosterone system (RAAS) has been associated with an increased risk of ischemic cardiovascular events and the use of angiotensin converting enzyme (ACE) inhibitors results in reduced rates of coronary thrombosis and recurrent myocardial infarction (1-3). The mechanism of this beneficial effect cannot be completely explained by the antihypertensive effects of ACE inhibitors. It may be related to the known property of angiotensin II (Ang-II) to increase plasminogen activator inhibitor type-1 (PAI-1) levels (4). Conceivably, ACE inhibitors would depress PAI-1 production. PAI-1 is the major physiological inhibitor of fibrinolysis (5) and increased levels of PAI-1 are associated with recurrent ischemic thrombotic attacks (6). Increased PAI-1 expression has also been demonstrated in atherosclerotic lesions (7).

Although a possible link between RAAS activation and ischemic cardiovascular events have been attributed to Ang-II (8), increasing evidence suggests that aldosterone also has harmful effects on the cardiovascular system and that aldosterone receptor blockers reduce cardiovascular mortality (9). The precise mechanisms by which aldosterone can cause cardiovascular injury have not been fully delineated but it is hypothesized that aldosterone may be responsible for vascular and myocardial fibrotic and hypertrophic effects independent of Ang-II (10). Moreover, recent data indicated that plasma aldosterone concentrations were positively correlated with plasma PAI-1 activity levels and aldosterone receptor antagonism abolishes plasma PAI-1 concentrations suggesting aldosterone may also regulate fibrinolysis (11).

On the other hand, results from recent studies have shown that the administration of an aldosterone receptor antagonist with an ACE inhibitor or an Ang-II receptor blocker (ARB) confers greater clinical benefit than the administration of an ACE inhibitor or an ARB alone (12,13). Although several potential mechanisms for the additive effects of aldosterone blockers either in combination with an ACE inhibitor or an ARB have been defined, the exact mechanism of this benefit is not well understood (9,10). We hypothesized that concurrent aldosterone receptor antagonism enhances the effect of RAAS inhibition (ACE or ARB) on fibrinolysis may contribute to this beneficial cardiovascular effect. Based on this hypothesis, we designed the present study to compare the acute effects of an ACE inhibitor and an ACE inhibitor in combination with aldosterone receptor antagonist on PAI-1 level in the setting of activation of the RAAS in healthy blood donors. We purposed to stimulate RAAS by phlebotomy, which is a fairly effective and physiologic method. We suspect that phlebotomy induced RAAS activation can cause reversible abnormalities in plasma levels of PAI-1.

Materials and Methods

Subjects

Seventeen male subjects (age, 27.9±4.1 years) meeting our inclusion criteria were recruited for this study from among the voluntary blood donors who presented to our university blood center over the past year. Only male subjects were selected, because the study was small and no value would be gained by including both sexes with so few subjects. Potentially eligible adults were selected in a way to achieve a certain degree of homogeneity in terms of age, normal laboratory values, blood pressure range, and other demographic variables (Table I). All potentially eligible subjects were evaluated in the outpatient setting. Medical history and physical examination were recorded for each subject, and each underwent tests for routine biochemical and hematological monitoring. The subjects were also asked for a 24-hour urine collection to measure sodium, creatinine, and volume. Those subjects who had significant cardiovascular, renal, endocrine (including diabetes) or pulmonary disease, or who were taking any medication were excluded. Subjects who had a history of smoking were also excluded.

Study Protocol

All persons gave informed consent to participate according to the ethical principles for human investigations as outlined in the second Declaration of Helsinki. Subjects were allowed to resume their usual diet. We investigated plasma renin activity (PRA), plasma aldosterone, and plasma PAI-1 activity levels before and after phlebotomy. Four months (mean; range 3-6 months) from the first phlebotomy, a second phlebotomy was performed in all subjects. Before the second phlebotomy, subjects were randomly divided into three groups. Group I (n=6, 6M, 27.6±3.6 years) received enalapril (5 mg/day p.o. for three days), group II (n=6, 6M, 28.2±4.8 y) received

Table I. Baseline clinical characteristics of all the subjects

Parameters	All subjects (n=17)	Group I (n=6)	Group II (n=6)	Group III (n=5)
Mean age (years)	27.9±4.1	27.6±3.6	28.2±4.8	27.8±3.4
Gender (males)	17	6	6	5
BMI, kg/m²	23.6±3.2	22.8±2.6	23.9±2.4	23.7±2.3
Mean SBP, mmHg	131.9±2.6	131.2±2.9	132.3±2.2	131.8±2.4
Mean DBP, mmHg	82.8±2.2	83.1±2.1	82.5±2.4	82.6±2.8
Fasting plasma cholesterol, mg/dL	154.24±64	150.32±36	157.84±58	156.32±46
Fasting plasma glucose, mg/dL	84.28±42	85.48±28	83.12±32	84.3±24
Plasma creatinine, mg/dL	0.82±0.16	0.80±1.12	0.84±0.24	0.82±0.46
Plasma uric acid, mg/dL	5.4±1.5	5.6±1.4	5.3±1.6	5.4±2.1
Urinary sodium (mEq/day)	177.32±64	180.53±48	175.22±84	178.48±56

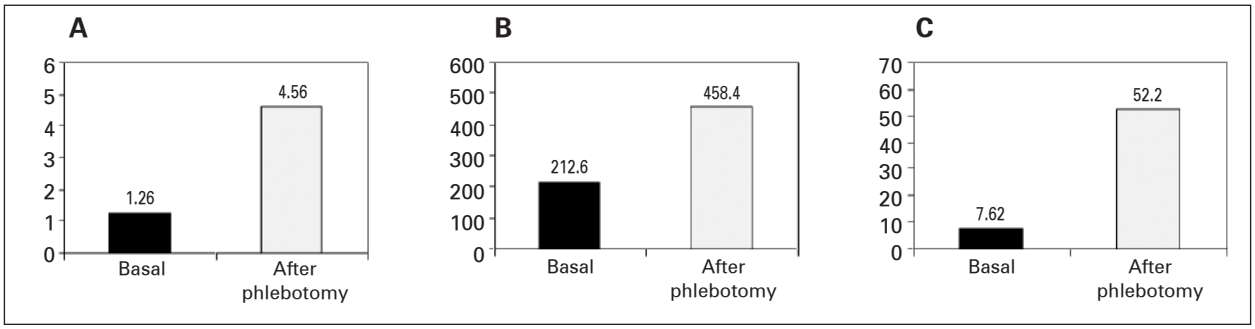

Figure 1. Mean PRA (ng/mL/h) (A), plasma aldosterone (pmol/L) (B) and PAI-1 activity (IU/mL) (C) levels before (basal) and after phlebotomy in all subjects (p<0.001, after phlebotomy vs. baseline)

enalapril [(5 mg/day p.o.) + spironolactone (25 mg/day p.o.), for three days)] and group III (n=5, 5M, 27.8±3.4 y) had no medications. Two hours after the last dose of medications, all subjects underwent a second phlebotomy. Blood samples were obtained for PRA, plasma aldosterone, and PAI-1 activity before and after phlebotomy. Continuous blood pressure and pulse monitoring was done during the study period.

Blood Samples

The study was a cross-sectional determination of PAI-1 activity in plasma samples taken from healthy blood volunteers. All samples were taken from patients in the fasting state before and soon after phlebotomy during the morning hours to avoid the effects of diurnal variation of the hemostatic system. After a 30-minute rest in the sitting position, blood samples were drawn from the large antecubital vein. All venipunctures were carried out without interruption of venous flow and with a 19-gauge butterfly needle connected to a plastic syringe. Fifteen milliliters of blood were drawn and the first few milliliters were discarded; next, 4.5 mL was transferred immediately to Stabilyte tubes (Biopool, Sweden) for determination of PAI-1 activity. Nine milliliters were transferred to polypropylene tubes containing 1 mL trisodium citrate (0.109 mmol/L) to determine the levels of other analytes. The tubes were then centrifuged at 3000 rpm for 15 min at 10-18°C. The supernatant plasma samples were stored in plastic tubes at -70°C until assayed.

Assays

PRA was estimated radioimmunologically (radioimmunoassay, Sorin, Renctz, CisBio Int., France), the reference (supine) range with normal salt intake being 0.2 to 5.7 ng/mL/h. Serum aldosterone was

assayed with a commercially available radioimmunoassay kit (Diagnostic Corporation). The intra- and interassay coefficients of variation were 6% and 10%, respectively. PAI-1 activity was measured by Chromogenic Assay (Biopool, Sweden). The intra- and interassay coefficients of variation ranged from 5.2% to 8.7% and from 6.5% to 9.4%, respectively.

Statistical Analysis

Differences in baseline plasma renin and PAI-1 levels before and after phlebotomy were evaluated by nonparametric Wilcoxon matched-pairs signed rank test. Within group and between group comparisons were performed to assess changes in variables between prephlebotomy and postphlebotomy, again using Wilcoxon matched-pairs signed rank test and two-way analysis of variance (ANOVA). Correlations were performed by Pearson correlation test. The data were analyzed using the statistical software program, SPSS (V11.0) for Windows (SPSS Inc.), and expressed as means ± standard deviations. P values lower than 0.05 were considered statistically-significant.

Results

Supine systolic and diastolic arterial blood pressure decreased in healthy blood donors after phlebotomy; however, it did not reach statistical significance ($p>0.05$). Following the first phlebotomy mean PRA, plasma aldosterone, and plasma PAI-1 activity levels increased significantly in all subjects ($p<0.001$) (Table II) (Figure 1). PRA and plasma aldosterone levels were also positively correlated with PAI-1 activity levels ($R=0.47$, $p=0.015$).

Mean systolic and diastolic blood pressure decreased significantly in the enalapril arm (group I) and enalapril + spironolactone arm (group II) after the second phlebotomy ($p<0.05$), and the magnitude of the reduction was similar in both groups ($p<0.05$). Mean systolic and diastolic blood pressures were also reduced in no medication arm (group III) after phlebotomy, but it did not reach statistical significance ($p=0.60$) (Table III; Figure 2).

The mean basal PRA, plasma aldosterone, and PAI-1 activity levels were not statistically different in the three groups ($p>0.05$) (Table III; Figure 3).

Table II. Mean blood pressure, PRA, plasma aldosterone, and PAI-1 activity levels before (baseline) and after first phlebotomy in all subjects

Parameters	Before phlebotomy	After phlebotomy	P values
SBP, mmHg	131.9±2.6	129.8±3.2	NS
DBP, mmHg	82.8±2.2	81.2±2.4	NS
PRA, ng/mL/h	1.26±0.80*	4.56±2.36*	<0.001
Aldosterone, pmol/L	212.6±26*	458.4±32*	<0.001
PAI-1 activity, IU/mL	7.62±3.86*	52.2±14.68*	<0.001

*p<0.001 after phlebotomy vs. before phlebotomy

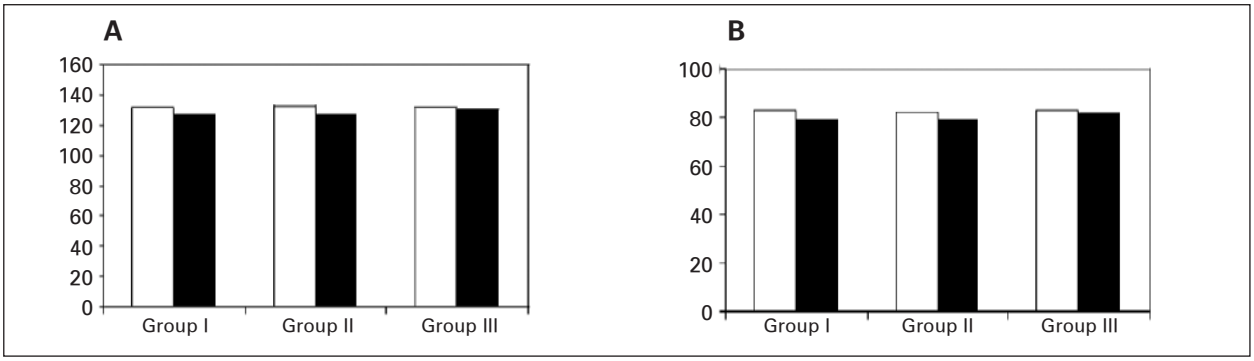

Figure 2. Mean systolic (A) and diastolic (B) blood pressure before and after phlebotomy in group I (enalapril), group II (enalapril+spironolactone), and group III (no medication).

Table III. Mean systolic blood pressure, diastolic blood pressure, PRA, plasma aldosterone, and PAI-1 activity levels before and after the second phlebotomy in three groups

Parameters	Group I (n=6) (Enalapril)		Group II (n=6) (Enalapril+Spironolactone)		Group III (n=5) (No medication)	
	Basal	Post-phlebotomy	Basal	Post-phlebotomy	Basal	Post-phlebotomy
SBP	131.2±2.9	126.4±4.2	132.3±2.2	126.8±2.8	131.8±2.4	130.2±1.8
DBP	83.1±2.1	79.6±3.6	82.5±2.4	79.2±2.2	82.6±2.8	81.8±2.2
PRA	1.24±0.78	8.52±2.2	1.28±1.1	8.22±2.1	1.26±0.56	4.38±2.16
Aldosterone	214.8±24	336.6±42	210.4±64	324.9±55	208.4±44	486.6±74
PAI-1 activity	7.28±2.8	28.6±9.2 &**	7.32±1.8	18.6±6.4 &**	7.88±3.4	48.24±9.4&

& p<0.01, group I and group II vs. group III
** p=0.024, group II vs. group I

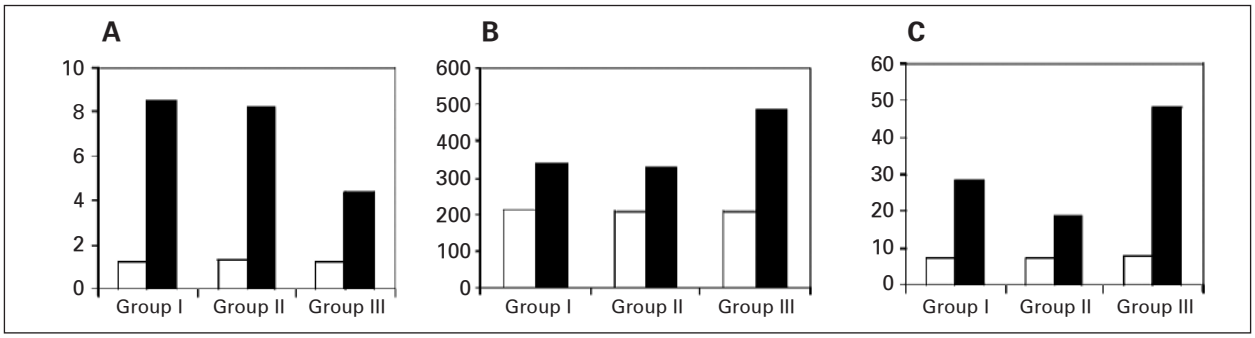

Figure 3. Mean PRA (ng/mL/h) (A), plasma aldosterone (pmol/L) (B) and PAI-1 activity levels before (basal) and after phlebotomy in group I (enalapril), group II (enalapril+spironolactone) and group III (no medication) (*PRA, plasma aldosterone, PAI-1 activity; &p<0.01, group I and group II vs. group III) (PAI-1 activity; *p=0.024, group II vs. group I)*

PRA increased significantly versus baseline and in response to phlebotomy in three groups (p<0.001); however, PRA increment in the 2 drug arms (group I and group II) was more marked than in group III (p<0.01). No significant difference in the levels of PRA was found between groups I and II in response to phlebotomy (p>0.05) (Table III) (Figure 3).

Plasma aldosterone levels increased significantly in three groups after phlebotomy (p<0.001). Plasma aldosterone increased more in group III than in groups I and II (p<0.01). The plasma aldosterone levels after phlebotomy were not statistically different in group I and group II (p>0.05) (Table III) (Figure 3).

Plasma PAI-1 activity levels also increased in response to phlebotomy in all groups (p<0.01). However, the levels of the plasma PAI-1 activity after phlebotomy were significantly lower in the enalapril (group I) and enalapril+spironolactone (group II) groups than in no medication group (group III) (p<0.01). Increased PAI-1 activity levels in response to phlebotomy were more suppressed by enalapril+spironolactone than by enalapril alone (p=0.024) (Table III; Figure 3).

Discussion

Strong evidence shows that fibrinolytic balance is largely under the control of the RAAS. Recent studies suggested that activation of the RAAS by salt depletion increases peak plasma PAI-1 concentrations, whereas ACE inhibition significantly decreases plasma PAI-1 antigen and activity level in hyperten-

sive and normotensive subjects (14). More recently, aldosterone receptor blockers have attained increased importance due to their beneficial angiotensin-independent effects on the cardiovascular system (10,12). Although the precise mechanism is unclear, an effect on fibrinolysis has been suggested (10). However, the effect of aldosterone antagonism on fibrinolysis has not been extensively studied in humans. Our study is the first to compare the acute effects of combination of ACE inhibition with aldosterone antagonism and ACE inhibition alone on fibrinolytic balance under conditions of controlled activation of the RAAS.

The present study, independent of blood pressure, demonstrated activation of the RAAS has been associated with increased PAI-1 activity levels. Recent studies have demonstrated that activation of the RAAS through either sodium depletion or diuresis increases plasma PAI-1 antigen and activity levels in healthy normotensive volunteers (14). Our findings are consistent with those of previous studies. However, it differs from similar previous studies in terms of RAAS activation method. In this study, RAAS was activated in a direct and efficient way by phlebotomy. In humans, a negative feedback system in which renal hypoperfusion controls renin production, consecutively, renin controls RAAS activation, provides homeostasis in blood pressure and oxygen delivery to tissues. As tissue hypoxia is provoked by phlebotomy, tissue oxygenation decreases and the levels of renin increase exponentially.

In our study, we showed that the mean PRA, plasma aldosterone, and PAI-1 activity levels of the healthy blood donors increased significantly after phlebotomy and that plasma PAI-1 activity levels were positively correlated with PRA and plasma aldosterone levels. Although the levels of blood pressure did not reach hypertensive stages in our study population, mean baseline systolic and diastolic blood pressure were higher than 120/80 mmHg, a prehypertension state. This finding is important since exaggerated PRA, aldosterone, and PAI-1 activity in response to phlebotomy might be related to the projection of an abnormal asymptomatic cardiovascular homeostasis. As shown in Table II, the pronounced exaggerated PAI-1 response to phlebotomy in prehypertensive subjects suggested a direct link between the RAA and fibrinolytic system in humans.

In the present study, administration of enalapril significantly reduced both exaggerated aldosterone and PAI-1 activity levels in response to phlebotomy, while PRA levels were elevated. These findings were consistent with previous studies conducted with ACE inhibition. Co-administration of spironolactone with enalapril reduced PAI-1 activity levels more than enalapril alone (Table III). Mean PRA and plasma aldosterone levels were similar in both groups. This additive effect of enalapril plus spironolactone on fibrinolysis was independent of blood pressure. The mean systolic and diastolic blood pressure levels were similar before and after phlebotomy in either enalapril or enalapril+spironolactone arm; thus, this result agrees with findings demonstrated in previous studies on the additional benefits of spironolactone with respect to fibrinolytic balance. One key difference between our study and prior reports is that we analyzed the acute effects of those drugs. This difference is critical in that it avoids the phenomenon of aldosterone escape, which is a physiological effect that occurs during long-term use of ACE inhibitors or angiotensin receptor blockers. Several prior studies had the potential limitation that treatment with an ACE inhibitor initially suppressed aldosterone levels; yet, aldosterone levels returned to baseline levels during treatment. This study clearly exhibited an additive contribution of spironolactone on fibrinolytic balance.

In conclusion, activation of the RAAS by phlebotomy is an effective and physiologic method. Increased PAI-1 activity in individuals with high-normal hypertension supports the hypothesis that RAAS plays a central role in fibrinolysis. Suppression of this effect occurs, with both the acute use of an ACE inhibitor and the use of a combination of ACE inhibitor and aldosterone blocker, the latter having a greater suppressant effect. This study provides evidence that endogenous aldosterone regulates PAI-1 production in humans. Aldosterone blockers, either modulating the effect on ANG II on PAI-1 expression (15) or acting as an angiotensin II independent mechanism (11), have additional effects on fibrinolytic system and may reduce the risk of vascular thrombotic events. However, neither ACE inhibitor alone nor the combination was sufficient to return PAI-1 levels to baseline. Additional prospective studies are needed to define the relationship and mechanism by which aldosterone antagonism and fibrinolysis take part.

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